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TOXICITY OF PERSISTENT CHEMICAL AGENT SIMULANTS (PCAS) AND CHEMICAL AGENT DISCLOSURE SOLUTION (CADS) IN SOIL ON CUCUMBER (Cucumis sativus, L.) AND EARTHWORMS (Eisenia foetida)

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13. ABSTRACT *(Maximum 200 words)*
Persistent chemical agent simulants (PCAS) of GD and HL and the chemical agent disclosure solution (CADS) were tested for their toxicity to cucumbers and earthworms. The PCAS and CADS were tested at 0, 0.10, 0.50, 1.0, and 2.5% concentration by weight. The GD simulant produced lethal and sublethal effects on cucumber plants at the 1.0% level. The HL simulant in soil on cucumbers was lethal above the 0.10% concentration. The CADS produced lethal and sublethal effects on cucumber plants at the 2.5% level. The analysis of covariance (ANCOVA) for earthworms treated with the GD simulant was not significant ($p < 0.05$) for weight differences; however, survival rates did decline with increasing concentrations. The HL simulant was the most toxic to earthworms with lethal effects being exhibited at the 0.05% level. The effects of CADS on earthworm weight change was not significant ($p < 0.05$) by ANCOVA between treatments through the 1.0% level. All earthworms died at the 2.5% level. The HL simulant was toxic to plants and earthworms at 0.10%, which is near the expected field-release level. The PCAS and CADS should not have an adverse effect on plant life if field-release levels are <0.10%. The GD simulant and CADS also should not affect soil organisms at these same levels.

14. SUBJECT TERMS
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PREFACE

The work described in this report was authorized under Project No. 89HE-02-001. This work was started in April 1989 and completed in May 1990.

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TOXICITY OF PERSISTENT CHEMICAL AGENT SIMULANTS (PCAS) AND CHEMICAL AGENT DISCLOSURE SOLUTION (CADS) IN SOIL ON CUCUMBER (*Cucumis sativus*, L.) AND EARTHWORMS (*Eisenia foetida*)

1. INTRODUCTION

There is a need to provide environmentally safe persistent chemical agent simulants (PCAS) that mimic blister and nerve agents and can be used in chemical warfare exercises. A simulant is also needed to replace the hazardous decontaminants during field training exercises. The chemical agent disclosure solution (CADS) is intended to determine the efficiency of simulant decontamination.

The purpose of this study was to develop baseline environmental toxicity data on the PCAS for GD (soman) and HL (mustard-lewsite) agents and CADS. This study was composed of phytotoxicity and earthworm toxicity testing. These test results will help determine at what point using these materials may adversely affect the environment. The data generated will be used in environmental documents and assist decision makers in determining the use of these materials.

The study of the toxic effects of chemicals on plants is important for several reasons. One reason is that chemicals may adversely affect plant development. In addition, chemicals may enter human food chains through processes associated with soil/plant interactions, uptake, translocation, and accumulation in food and forage crops. The type of phytotoxicity testing employed in these studies uses plant height measurements as plant growth indicators.

The second phase of this study involved earthworm toxicity testing. Earthworms, because of their role in maintaining the physical characteristics and processes of soil (e.g., aeration, water permeability, and breakdown of organic matter) are considered key organisms in the soil community. These organisms increase the fertility of soil by increasing the availability of nutrients and are also an important link in the food chain. Earthworms can number up to 250 K individuals/acre. Roberts and Dorough[1] published a review of the importance of earthworms to terrestrial ecosystems and their use in assessing the hazards of chemicals to these nontarget organisms. Dean-Ross[2] discusses the strengths and weaknesses of experimental methods for testing the toxicity of chemicals to earthworms and the sensitivity of earthworm species to various chemicals. Based on this review, Dean-Ross recommended that *Lumbricus terrestris* and *Eisenia foetida* be used as the test species of choice.

2. METHODS AND MATERIALS

2.1 PCAS Simulation of GD.

A PCAS simulant was developed to emulate the physical properties of GD, a persistent nerve agent. The GD simulant composition is contained in Table 1.

Table 1. Characteristics of GD Simulant

Constituent	Function	Percentage (by weight)
Sodium Carbonate	Provide pH Cue	1.98
Polyethylene Oxide	Viscoelastic Thickener	0.99
Hydroxy Ethyl Cellulose	Stabilizer & "Scrubbability"	0.35
Glycerol	Plasticizer	9.91
Diethyl Malonate	Chemical Trigger	13.00
Water	Solvent and Extender	73.77

2.2 PCAS Simulation of HL.

The HL simulant was developed to mimic the physical properties of the HL agent and to be used during field exercises. The HL simulant composition is given in Table 2.

Table 2. Characteristics of HL Simulant

Constituent	Function	Percentage (by weight)
Ferrous Ammonium Sulphate	Provides Fe^{++} Cue	1.98
Polyethylene Oxide	Viscoelastic Thickener	0.25
Hydroxy Ethyl Cellulose	Stabilizer & "Scrubbability"	0.35
Glycerol	Plasticizer	9.91
Methyl Salicylate	Chemical Trigger	13.00
Water	Solvent and Extender	74.51

2.3 CADS.

The CADS will be applied to surfaces that have been subjected to PCAS "contamination" and have undergone decontamination processes. CADS visually discloses whether any PCAS residue remains on the decontaminated surfaces. Table 3 lists the components of CADS.

Table 3. Chemical Agent Disclosure Solution (CADS)

Constituent	Function	Percentage (by weight)
2,2-Dipyridyl	Turns Red in Presence of Fe^{++}	0.50
Phenolphthalein	Turns Red in Presence of Na_2CO_3	1.00
Isopropanol	Solvent for Indicators	70.00
Distilled Water	Extender	28.50

2.4 Plant Study.

The test methods used for phytotoxicity studies were adapted from the U.S. Environmental Protection Agency's (USEPA) Environmental Effects Test Guidelines (Early Seedling Growth Toxicity Test).[3] The plant species used in this study, chosen from the USEPA's list of recommended crops, was cucumber [*Cucumis sativus* L., cv. Straight Eight]. Cucumber seeds were obtained from the Meyer Seed Company (Baltimore, MD) and sorted to ensure uniform size. Damaged and malformed seeds were discarded.

The soil used for these tests was obtained from a site at M-Field, Aberdeen Proving Ground, Edgewood Area, MD. The soil was a moderately eroded acidic Sassafras sandy loam [fine-loamy, siliceous, mesic Typic Hapludult], yielding a loamy sand texture. The respective physical and chemical properties of the soil are given in Table 4.

Table 4. Physical and Chemical Characteristics of M-Field Soil

Soil Parameters*		
Mechanical Analysis	Soil Analysis	
% sand	NO_3 (lb/A) -	9.2
87	P_2O_5 (lb/A) -	14.0
	K_2O (lb/A) -	48.0
% silt	Ca (lb/A) -	10.0
9	Mg (lb/A) -	24.0
	Mn (lb/A) -	5.0
% clay	Zn (lb/A) -	2.0
4	Cu (lb/A) -	1.8
	CEC (meq/100 g) -	2.2
% organic matter - 0.3	pH -	4.6
Texture - sandy loam		

*Determined by the Soil Testing Laboratory, University of Maryland (College Park, MD)

The soil was air-dried and seived to pass a 2-mm screen. The test solution was mixed with a small amount of M-Field soil (on a weight basis) to produce a spike, which was then mixed with additional M-Field soil to achieve the desired concentration. The same concentrations of GD and HL simulants and the CADS used in each independent study were 0, 0.10, 0.50, 1.0, and 2.5%. In each experiment for each concentration used, individual treatment pots were prepared in triplicate by placing a 10-cm^2 piece of cheesecloth in the bottom of each 15-cm flower pot, followed by 1,300 g of pea gravel (3-5 mm diameter). A second piece of moistened cheesecloth was placed on top of the pea gravel. The spiked soil/soil mixture (Table 5) was mixed in a Hobart (Hobart Corporation, Troy, OH) food blender and then added to the pots. Twenty seeds were planted to a depth of 5 mm in each pot. Pots were watered on a weight basis to provide 18% moisture. The experimental design (Table 5) for the PCAS and the CADS was a complete randomized block design with blocks of treatments replicated in triplicate.

Table 5. Experimental Treatments for Plant Study

Test Concentration	Flower Pot No.	Spiked Soil:Soil (g)
Control (0%)	1, 2, 3*	0:1000
0.10%	4, 5, 6	1:999
0.50%	7, 8, 9	5:995
1.00%	10, 11, 12	10:990
2.50%	13, 14, 15	25:975

*Replicates in rows (e.g., 1, 2, and 3), blocks in columns (e.g., 1, 4, 7, 10, and 13), pots randomized within each block

Individual treatment pots within blocks were randomized once a week for the 2-week growth period. The pots within each block were rotated 180° three times per week because of cucumber plants' phototropic response to the sun.

After 50% of the control seedlings had emerged, the plants were thinned to the 10 most uniform plants in each pot. The plants were grown for 14 days following emergence (day 1). Plant heights were measured in situ twice per week following thinning, and a final measurement was made at harvest on day 14. Plant data were statistically analyzed using the two-way Analysis of Variance (ANOVA) and the Newman-Keuls pairwise comparison of means.[4]

2.5 Earthworm Study.

The method determining the toxicity of contaminants in soil to the earthworm, developed by Karnak and Hamelink,[5] was adapted to determine the toxicity of PCAS and CADS in soil. The earthworms used in the experiments were *Eisenia foetida* [Bert's Bait (Irvine, KY)]. The earthworms were housed in an incubator (13.0 ± 0.2 °C) during the course of the study.

The test media consisted of a nonsterile artificial soil and distilled water. The use of an artificial soil limits test variability that would otherwise occur due to heterogeneity of soil parameters. Other advantages of using an artificial soil mixture are ease of preparation and comparability to other data in the literature.[6] The components of the artificial soil follow:

Finely ground sphagnum peat	10%
Kaolinite clay	20%
Fine sand	69%
Calcium carbonate	1%

For each replicate, 200 g of artificial soil was used. The test solution was mixed with distilled water volumetrically to provide the concentration of PCAS or CADS needed. The liquid was then added to the soil and mixed in a food blender for 3 min until thoroughly blended. The soil moisture level was 25% of the total soil used. The concentrations of PCAS or CADS in the soil were the same as the concentrations in the plant study (i.e., 0, 0.10, 0.50, 1.0, and 2.5%). The test soil was placed in a 600-mL beaker. Five earthworms were weighed as a group, and groups were randomly added to each beaker, which was covered with nylon screen and cheesecloth held in place by a rubber band. The beakers were randomly placed in a precision low-temperature incubator (GCA Corporation, Precision Scientific Group, Chicago, IL) at 13.0 ± 0.2 °C. After 14 days, the earthworms in each beaker were weighed again and examined for physical condition. Three replicates per concentration were used for each experiment. The experimental design (Table 6) was a randomized complete block.

Table 6. Experimental Treatments for Earthworm Study

Test Concentration	Beaker #	Soil (g)	Simulant:Water (mL)
Control (0%)	1, 2, 3*	200.0	0.0:50.0
0.10%	4, 5, 6	200.0	0.2:49.8
0.50%	7, 8, 9	200.0	1.0:49.0
1.00%	10, 11, 12	200.0	2.0:48.0
2.50%	13, 14, 15	200.0	5.0:45.0

*Replicates in rows (e.g., 1, 2, and 3), blocks in columns (e.g., 1, 4, 7, 10, and 13), beakers randomized within each block

The Newman-Keuls pairwise comparison of means and the Analysis of Covariance (ANCOVA)[7] were the statistical methods used to evaluate the earthworm data.

3. RESULTS AND DISCUSSION

3.1 Effects of PCAS and CADS on Plants.

The average plant height for cucumbers at harvest, 14 days after emergence, is given in Figure 1 for the GD simulant, Figure 2, for the HL simulant, and Figure 3 for the CADS treatment. Figure 4 shows a comparison of the effects of each solution on mean plant heights.

The heights of the cucumber plants treated with GD simulant are given in Table A-1. The two-way ANOVA of these plants' heights indicated a significant ($p < 0.01$) difference between treatments, with no significant ($p < 0.05$) difference between blocks (Table B-1). The Newman-Keuls Test (Table B-4) was performed to determine whether the differences in plant heights were due to treatment or other factors. The test showed no significant ($p < 0.05$) difference between the control group and the 0.10% level. However, the difference between the 0.50% level and either the control group or the

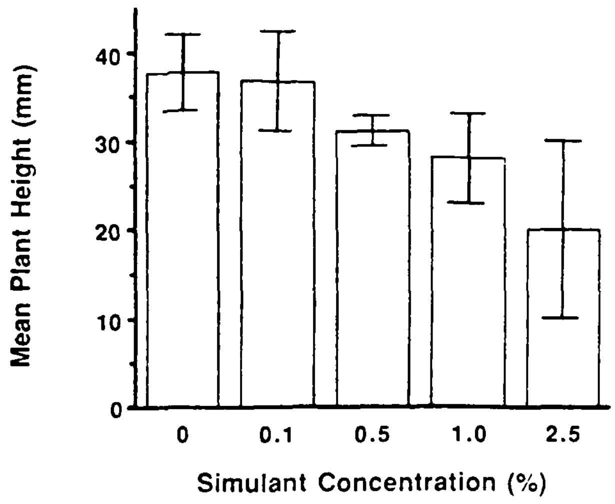

Figure 1. Effects of GD Simulant in Soil on the Mean Plant Height of Cucumber

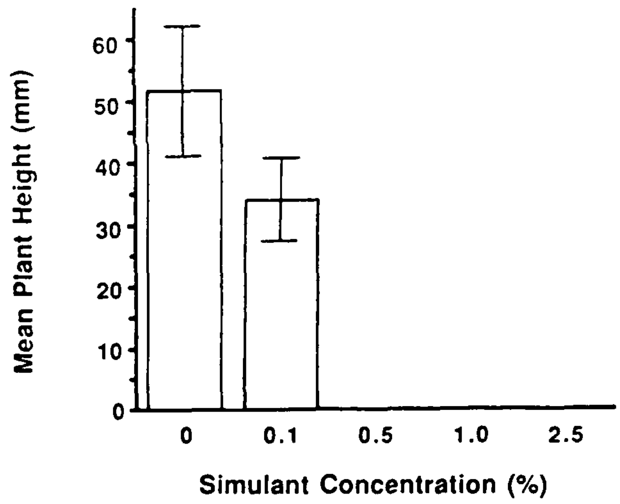

Figure 2. Effects of HL Simulant in Soil on the Mean Plant Height of Cucumber

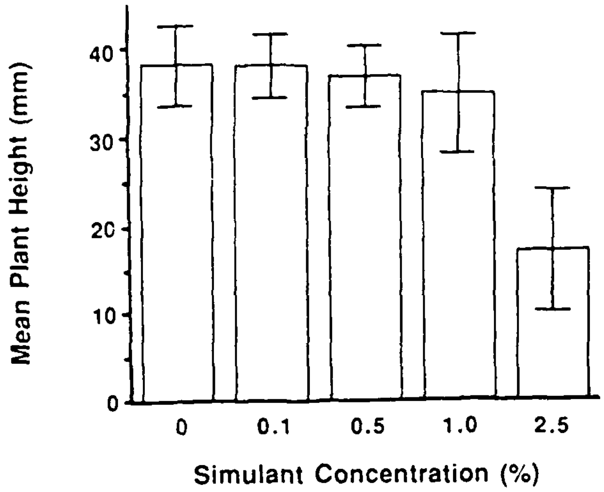

Figure 3. Effects of CADS in Soil on the Mean Plant Height of Cucumber

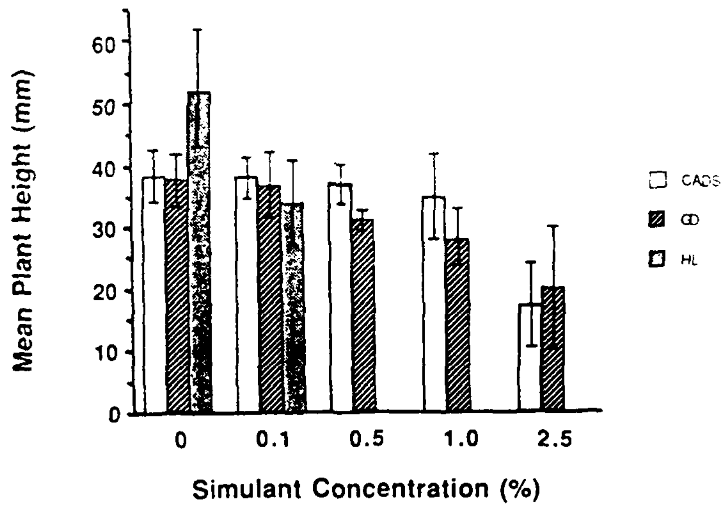

Figure 4. Comparison of the Effects of PCAS/CADS in Soil on Cucumber Plants

0.10% level was significant at $p < 0.01$. Survival rates of cucumber plants grown in soil amended with the GD simulant are given in Figure 5. The survival rate was 100% for the 0, 0.10, and 0.50% levels. The rate was 23% at the 1.0% level and dropped to 10% at the 2.5% concentration.

The test of HL simulant in soil on cucumbers resulted in survival of only the control group and the 0.10% group (Figure 6 shows the survival rates at the 0% and 0.10% levels with no plants surviving at higher concentrations; compare this with Figure 2, the mean plant heights). The HL simulant was lethal to the plants at a concentration of 0.50% or higher. The ANOVA (Table B-2) for the 0 and 0.10% levels was significant at $p < 0.01$. The mean plant height was 52.2 ± 10.4 mm for the control and 34.6 ± 7.0 mm for the 0.10% level (Table A-2).

The CADS in soil produced lethal and sublethal effects on cucumber plants at 2.5%, only 57% of the plants survived at this level (Figure 7). There was a significant ($p < 0.05$) difference between treatments (Table B-3), but there was not a significant ($p < 0.05$) difference between blocks. A Newman-Keuls Test (Table B-5) of plant heights was significant ($p < 0.05$) between the control group and the 1.0% group. This test indicated that the CADS was beginning to produce a negative effect on plant heights at this concentration. Plants grown at the 2.5% concentration exhibited the most lethal and sublethal effects; of the 60 seeds sown (20 per pot), only 17 plants survived at this concentration. The average plant height for this level was 17.1 ± 6.9 mm, compared with an average height of 34.7 ± 6.9 mm at the 1.0% level (Table A-3).

Figure 8 provides a comparison of the effects that PCAS and CADS had on survival rates of cucumbers. At concentrations through the 0.10% level, 100% of the plants grown in soil amended with either PCAS or CADS survived. The GD simulant and CADS had a 100% survival rate at the 0.50% level, but the HL simulant had no survivors at this concentration or at higher levels. Increasing the concentration to 1.0% reduced the GD simulant survivorship to 23%; whereas, the CADS survival rate remained at 100%. At the 2.5% level, the CADS survival rate was reduced to 57%, and the GD simulant survival rate was only 10%. These results indicate that the HL simulant exhibits toxic effects at lower concentrations than does either the GD simulant or the CADS.

3.2 Effects of PCAS and CADS on Earthworms.

The ANCOVA (Table D-1) for earthworms treated with GD simulant showed that the difference between the beginning weight and the ending weight was not significant ($p < 0.05$) among the various treatment levels. Although the average weight difference among the treatment levels was not significant for the ANCOVA, the Newman-Keuls Test was significant ($p < 0.05$) between the 2.5 and 0-0.50% levels (Table D-4). Survival rates did decline with increasing concentrations (Figure 9). In the control and 0.10% groups, all of the earthworms survived. The survival rate dropped to 86.7% for the 0.50 and 1.0% treatments, and at 2.5% concentration, 80% of the earthworms survived (Table C-1).

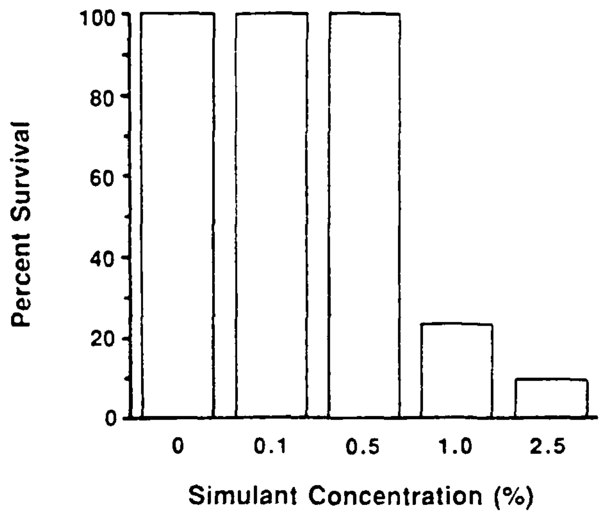

Figure 5. Survival of Cucumber Plants Grown in Soil Amended with GD Simulant

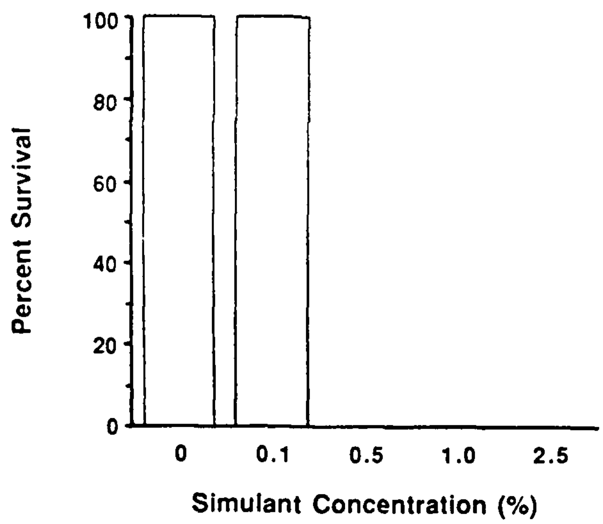

Figure 6. Survival of Cucumber Plants Grown in Soil Amended with HL Simulant

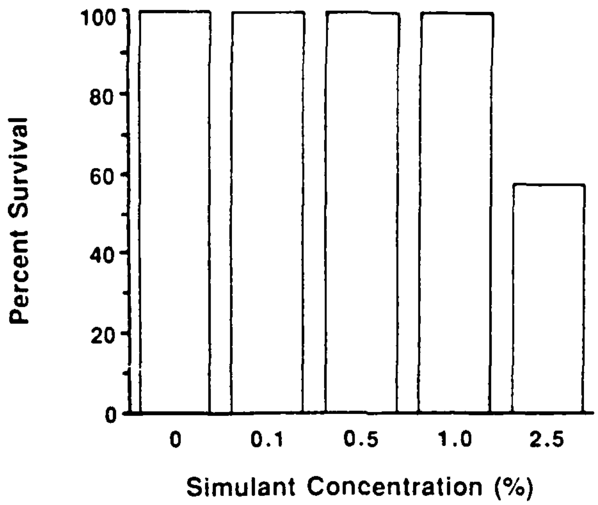

Figure 7. Survival of Cucumber Plants Grown in Soil Amended with CADS

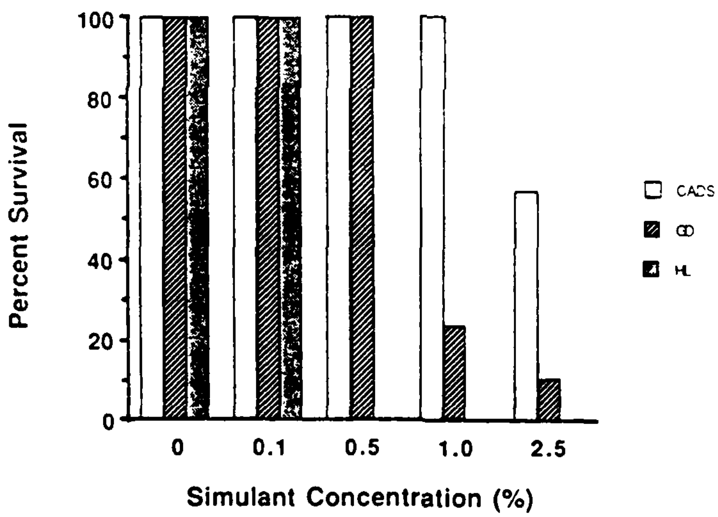

Figure 8. Comparison of the Lethal Effects of PCAS/CADS on Cucumbers in Soil

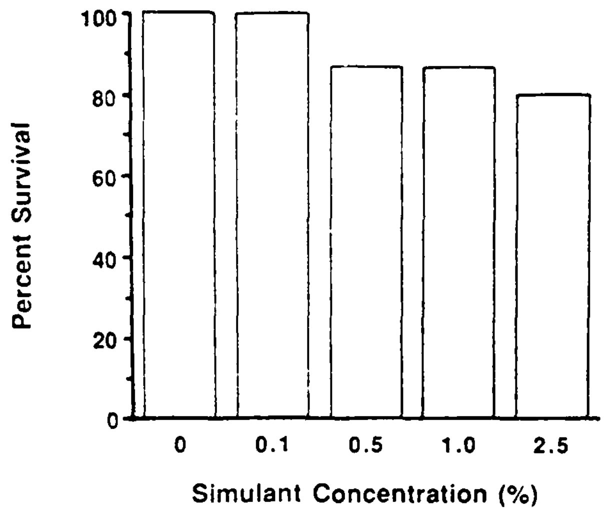

Figure 9. Survival Rate of Earthworms in Soil Amended with GD Simulant

The HL simulant was the most toxic to earthworms. In the control group, all of the earthworms survived, but at the 0.10% concentration, 20% of the earthworms survived (Figure 10), and in concentrations above the 0.10% level, no earthworms survived. The ANCOVA was not significant at $p < 0.05$ (Table D-2). The Newman-Keuls pairwise comparison (Table D-2) of mean earthworm weights gave a significant ($p < 0.05$) difference between the 0.10% HL treatment, in which earthworms lost weight, and the controls, in which earthworms gained weight (Table C-2).

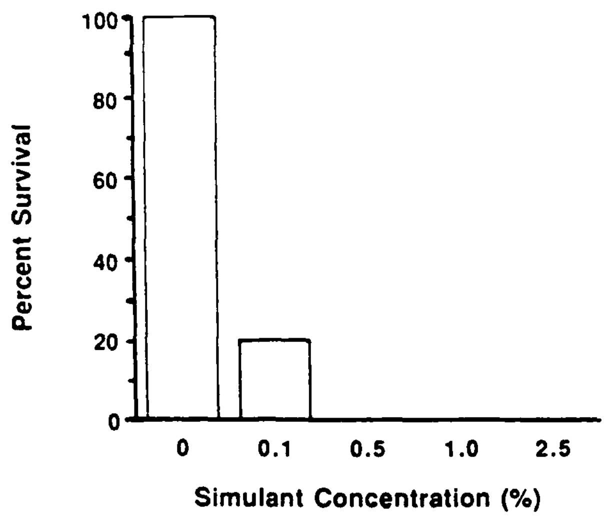

Figure 10. Survival Rate of Earthworms in Soil Amended with HL Simulant

The ANCOVA (Table D-3) statistical analysis of the weight change data for CADS-treated earthworms indicated no significant ($p < 0.05$) difference among treatments through the 1.0% level. The Newman-Keuls (Table D-6) analysis indicated a significant ($p < 0.05$) difference between the 0.50% level and the

0, 0.10, and 1.0% levels. All of the earthworms died at the 2.5% concentration (Figure 11). These results indicate that acute toxicity of CADS to earthworms begins at a concentration between 1.0% and 2.5% (Table C-3).

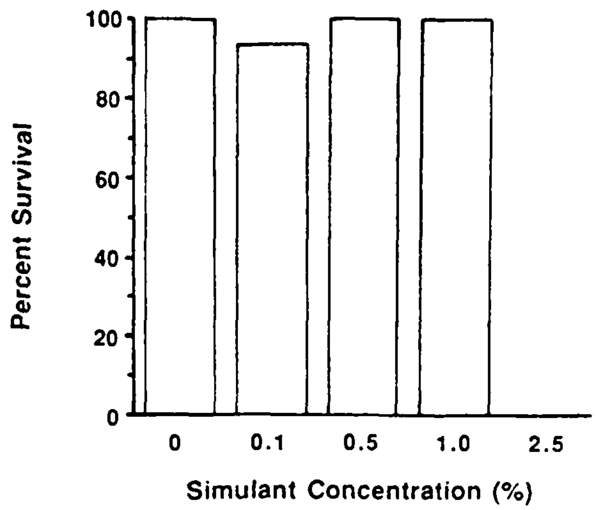

Figure 11. Survival Rate of Earthworms in Soil Amended with CADS

Figure 12 shows a comparison of the survival rates of earthworms in soil amended with PCAS or CADS, respectively. The survival rate was 20% for earthworms raised in soil amended with the HL simulant at 0.10% level; whereas, the survival rate for higher HL simulant concentrations was 0%. The earthworm survival rate for the GD simulant was 100% at the 0.10% level. The survival rates dropped to 86.7% at the 0.50 and the 1.0% levels, and at the 2.5% level, the survival rate was 80%. The survival rate for CADS was 93% at the 0.10% level, 100% at the 0.50 and 1.0% levels, 0% at the 2.5% level.

These results indicate that at lower concentrations, the HL simulant is more toxic than either the GD simulant or the CADS, with lethal and sublethal effects of HL simulant being exerted at the 0.10% level. Deleterious effects of the GD simulant on earthworm survival began at the 0.50% level. For CADS, adverse survival rates were produced at the 2.5% concentration.

4. CONCLUSIONS

The GD simulant produced lethal and sublethal effects on cucumber plants at the 1.0% level. There was also a significant ($p < 0.01$) difference in mean plant heights between the control group and the 0.50% level. The GD simulant produced toxic effects on earthworms at the 2.5% level. Earthworm survival rates at the 0.50 and 1.0% levels were 86.7% for both groups, and survival rates dropped to 80% at the 2.5% level. These results indicate that GD simulant should not have an adverse effect on the environment if field-release concentrations do not exceed 0.10% by weight in soil.

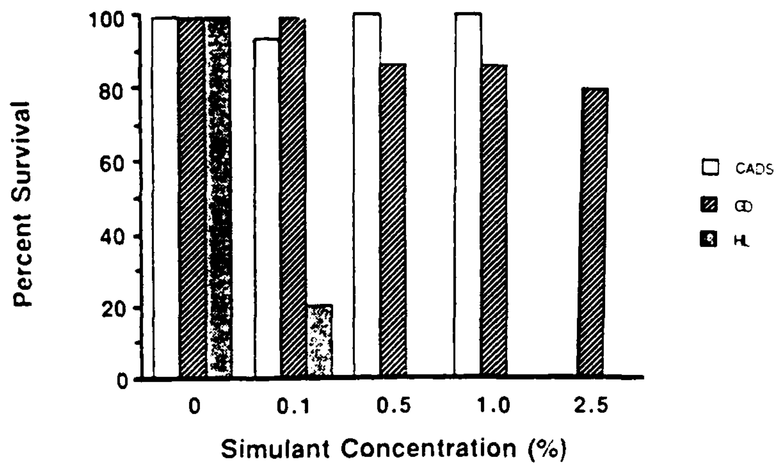

Figure 12. Comparison of Earthworm Mortality in Soil Amended with PCAS/CADS

The HL simulant produced lethal and sublethal effects on cucumber plants at the 0.10% level. There was a significant ($p < 0.01$) difference in mean plant heights between the control treatment and the 0.10% concentration. The effects on earthworms were similar to those on plants. The HL simulant reduced earthworm survival rates to 20% at the 0.10% level. All earthworms died at higher concentrations. The use of HL simulant in field-training exercises may have a negative environmental impact on plants and soil organisms when field-release levels in the soil are at or near the 0.10% concentration.

The results of CADS toxicity testing on plants and earthworms were consistent for both studies. The CADS produced lethal or sublethal effects on both test organisms only at the highest concentration tested (i.e., the 2.5% level), which should be well above any intended field-release level. Therefore, CADS should have a negligible impact on the environment.

Additional research should be conducted on the HL simulant to determine what the full impact will be on the ecosystem when this compound is released into the environment in its present formulation, since the HL simulant produced toxic effects on plants and earthworms at the 0.10% concentration (near field-release levels). Another approach to studying the toxicity of HL simulant in the environment is to determine if a single component of the HL simulant produced the reported toxic effects. Additional development may then allow replacement with an ingredient that is less toxic at field-release levels.

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APPENDIX A

PLANT DATA

Heights of Cucumber Plants Grown in Soil
Amended with Either PCAS or CADS

Table A-1. Heights of Cucumber Plants on Day 14, Grown in Soil Amended with GD Simulant

		Plant Heights (mm)				
Replicate No.	Plant No.	0%	0.10%	0.50%	1.0%	2.5%
I	1	37	29	31	17	-
	2	42	33	32	33	-
	3	34	37	32	29	-
	4	33	31	30	25	-
	5	39	34	33	32	-
	6	44	42	32	-	-
	7	36	41	31	-	-
	8	37	39	32	-	-
	9	41	40	30	-	-
	10	31	35	34	-	-
					-	
II	1	35	37	30	30	24
	2	42	35	33	-	9
	3	37	43	29	-	-
	4	31	47	32	-	-
	5	36	38	30	-	-
	6	41	43	31	-	-
	7	41	38	34	-	-
	8	34	33	27	-	-
	9	41	25	31	-	-
	10	30	34	30	-	-
III	1	37	40	29	29	27
	2	33	41	27	-	-
	3	40	41	34	-	-
	4	45	37	33	-	-
	5	36	49	31	-	-
	6	33	29	30	-	-
	7	45	32	30	-	-
	8	36	35	32	-	-
	9	43	35	31	-	-
	10	41	29	33	-	-
Mean:		37.7	36.7	31.1	27.9	20.0
Std. Dev.:		4.3	5.5	1.8	5.4	9.6

Table A-2. Heights of Cucumber Plants on Day 14, Grown in Soil Amended with HL Simulant

		Plant Heights (mm)				
Replicate No.	Plant No.	0%	0.10%	0.50%	1.0%	2.5%
I	1	48	35	-	-	-
	2	66	26	-	-	-
	3	69	34	-	-	-
	4	76	35	-	-	-
	5	46	34	-	-	-
	6	62	34	-	-	-
	7	55	42	-	-	-
	8	64	36	-	-	-
	9	39	31	-	-	-
	10	46	41	-	-	-
II	1	46	25	-	-	-
	2	42	32	-	-	-
	3	41	33	-	-	-
	4	52	34	-	-	-
	5	54	40	-	-	-
	6	63	23	-	-	-
	7	52	39	-	-	-
	8	48	29	-	-	-
	9	56	32	-	-	-
	10	46	26	-	-	-
III	1	50	23	-	-	-
	2	52	37	-	-	-
	3	35	34	-	-	-
	4	69	34	-	-	-
	5	57	35	-	-	-
	6	49	39	-	-	-
	7	49	43	-	-	-
	8	32	31	-	-	-
	9	57	46	-	-	-
	10	46	56	-	-	-
Mean:		52.2	34.6			
Std. Dev.:		10.4	7.0			

Table A-3. Heights of Cucumber Plants on Day 14, Grown in Soil Amended with CADS Simulant

Replicate No.	Plant No.	Plant Heights (mm) 0%	0.10%	0.50%	1.0%	2.5%
I	1	38	32	34	28	16
	2	38	38	34	25	10
	3	40	39	40	29	3
	4	38	45	34	44	18
	5	44	35	40	38	31
	6	45	33	36	35	14
	7	38	35	37	23	-
	8	36	40	35	28	-
	9	44	43	42	30	-
	10	35	39	35	30	-
II	1	34	35	42	42	8
	2	35	38	35	33	17
	3	40	45	36	44	16
	4	33	36	39	47	22
	5	35	42	42	47	18
	6	43	40	40	32	-
	7	36	37	37	44	-
	8	35	42	39	40	-
	9	43	42	34	38	-
	10	42	38	38	27	-
III	1	39	40	33	37	27
	2	37	35	33	35	22
	3	37	38	29	35	15
	4	30	39	32	40	11
	5	35	35	34	38	22
	6	30	38	36	32	20
	7	45	37	35	35	-
	8	43	38	36	36	-
	9	44	35	44	26	-
	10	33	31	40	24	-
Mean:		38.2	38.0	36.7	34.7	17.1
Std. Dev.:		4.4	3.5	3.5	6.9	6.9

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APPENDIX B

STATISTICAL DATA - PLANTS

Analysis of Variance (ANOVA) of Heights of Cucumber Plants Grown in Soil Amended with Either PCAS or CADS
and
Newman-Keuls Analysis of Treatment of Cucumber Plant Heights Grown in Soil Amended with Either PCAS or CADS

Table B-1. Analysis of Variance Two-Way, Single Observation: Plant Height (mm) of Cucumber (10 Plants/Block), Grown in Soil Amended with GD Simulant

Parameter	Control 0%	0.10%	0.50%	1.0%	2.5%
N:	30.000	30.000	30.000	-	-
Mean:	37.700	36.700	31.100	-	-
Std. Dev.	4.300	5.489	1.814	-	-

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	2259.797	89	-	-
Treatments:	754.164	2	377.082	21.84
Blocks:	504.453	29	17.395	1.01
Within:	1001.180	58	17.262	-

F (95%): 3.23 F (99%): 5.18
Differences Between Treatments: Significant at $p < 0.01$

F (95%): 1.89 F (99%): 2.47
Differences Between Blocks: Not Significant at $p < 0.05$

Table B-2. Analysis of Variance Two-Way, Single Observation: Plant Height (mm) of Cucumber (10 Plants/Block), Grown in Soil Amended with HL Simulant

Parameter	Control 0%	0.10%	0.50%	1.0%	2.5%
N:	30.000	30.000	-	-	-
Mean:	52.233	34.633	-	-	-
Std. Dev.	10.348	6.985	-	-	-

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	9166.731	59	-	-
Treatments:	4646.399	1	4646.39900	58.52
Blocks:	2217.735	29	76.47400	0.96
Within:	2302.599	29	79.39996	-

F (95%): 4.18 F (99%): 7.60
Differences Between Treatments: Significant at $p < 0.01$

F (95%): 1.90 F (99%): 2.49
Differences Between Blocks: Not Significant at $p < 0.05$

Table B-3. Analysis of Variance Two-Way, Single Observation: Plant Height (mm) of Cucumber (10 Plants/Block), Grown in Soil Amended with CADS Simulant

Parameter	Control 0%	0.10%	0.50%	1.0%	2.5%
N:	30.000	30.000	30.000	30.000	-
Mean:	38.167	38.000	36.700	34.733	-
Std. Dev.	4.371	3.503	3.485	6.887	-

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Total:	2864.7710	119	-	-
Treatments:	226.4375	3	75.479	3.46
Blocks:	742.7969	29	25.614	1.18
Within:	1895.5370	87	21.788	-

F (95%): 2.76 F (99%): 4.13
Differences Between Treatments: Significant at $p < 0.05$

F (95%): 1.89 F (99%): 2.47
Differences Between Blocks: Not Significant at $p < 0.05$

Table B-4. Newman-Keuls Analysis of all Treatments, Pairwise, and Ranked from Low to High: Cucumber Plant Heights (mm), Grown in Soil Amended with GD Simulant

Treatment	2.5%	1.0%	0.50%	0.10%	0%
			q values		
2.50%		3.631*	5.863*	8.812**	9.321**
1.00%			2.489	6.743	7.478**
0.50%				6.916	8.110**
0.10%					1.194
0.00%					
q(95%)		2.81	3.38	3.71	3.95
q(99%)		3.73	4.23	4.54	4.76

*Significant at $p < 0.05$
**Significant at $p < 0.01$

Table B-5. Newman-Keuls Analysis of all Treatments, Pairwise, and Ranked from Low to High: Cucumber Plant Heights (mm), Grown in Soil Amended with CADS

Treatment	2.5%	1.0%	0.50%	0.10%	0%
			q values		
2.50%		16.230**	18.036**	19.230**	19.383**
1.00%			2.123	3.527*	3.707*
0.50%				1.404	1.584
0.10%					0.180
0.00%					
q(95%)		2.77	3.31	3.63	3.86
q(99%)		3.64	4.12	4.40	4.60

*Significant at $p < 0.05$
**Significant at $p < 0.01$

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APPENDIX C

EARTHWORM DATA

Weight Differences (±) of Earthworms in Soil Amended with Either PCAS or CADS

Table C-1. Weight Differences (±) of Earthworms in Soil Amended With GD Simulant

Percent of GD sim.	No. of initial earth-worms	Average initial weight (g)	No. of final earth worms	Average final weight (g)	Mean initial weight (g)	Mean final weight (g)	Net weight change (±)
	5	0.21	5	0.32			
0.00	5	0.24	5	0.37	0.23	0.32	+0.09
	5	0.23	5	0.27			
	5	0.24	5	0.31			
0.10	5	0.23	5	0.31	0.24	0.31	+0.07
	5	0.25	5	0.32			
	5	0.24	5	0.32			
0.50	5	0.20	5	0.28	0.22	0.30	+0.08
	5	0.22	3	0.31			
	5	0.26	5	0.39			
1.00	5	0.27	4	0.33	0.26	0.37	+0.11
	5	0.25	4	0.39			
	5	0.23	3	0.38			
2.00	5	0.30	5	0.43	0.26	0.40	+0.14
	5	0.25	4	0.38			

Table C-2. Weight Differences (±) of Earthworms in Soil Amended with HL Simulant

Percent of HL sim.	No. of initial earth-worms	Average initial weight (g)	No. of final earth-worms	Average final weight (g)	Mean initial weight (g)	Mean final weight (g)	Net weight change (±)
	5	0.31	5	0.41			
0.00	5	0.26	5	0.33	0.29	0.37	+0.08
	5	0.30	5	0.37			
	5	0.24	0	0.00			
0.10	5	0.28	2	0.28	0.26	0.21	-0.05
	5	0.26	1	0.34			
	5	0.30	0	0.00			
0.50	5	0.30	0	0.00	0.28	0.00	0.00
	5	0.24	0	0.00			
	5	0.25	0	0.00			
1.00	5	0.24	0	0.00	0.26	0.00	0.00
	5	0.30	0	0.00			
	5	0.24	0	0.00			
2.50	5	0.25	0	0.00	0.25	0.00	0.00
	5	0.25	0	0.00			

Table C-3. Weight Differences (±) of Earthworms in Soil Amended with CADS

Percent of CADS	No. of initial earth-worms	Average initial weight (g)	No. of final earth-worms	Average final weight (g)	Mean initial weight (g)	Mean final weight (g)	Net weight change (±)
	5	0.14	5	0.28			
0.00	5	0.22	5	0.28	0.20	0.28	+0.08
	5	0.25	5	0.29			
	5	0.20	5	0.30			
0.10	5	0.23	5	0.28	0.22	0.28	+0.06
	5	0.23	5	0.26			
	5	0.19	5	0.21			
0.50	5	0.22	5	0.27	0.20	0.22	+0.02
	5	0.19	3	0.19			
	5	0.18	5	0.26			
1.00	5	0.26	4	0.28	0.24	0.27	+0.03
	5	0.27	4	0.28			
	5	0.21	3	0.00			
2.50	5	0.25	5	0.00	0.23	0.00	0.00
	5	0.22	4	0.00			

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APPENDIX D

STATISTICAL DATA - EARTHWORMS

Analysis of Covariance (ANCOVA) of Weight Differences of Earthworms Raised in Artificial Soil Amended with Either PCAS or CADS

Table D-1. ANCOVA of Weight Differences (g) of Earthworms in Soil Amended with GD Simulant

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F Value	Significance Level
Total:	0.01528	13			
Between:	0.00712	4	0.00178	1.96131	0.18438*
Within:	0.00816	9	0.00091		

*Not significant at p < 0.05

Table D-2. ANCOVA of Weight Differences (g) of Earthworms in Soil Amended with HL Simulant

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F Value	Significance Level
Total:	0.04612	4			
Between:	0.00331	1	0.00331	0.23214	0.66291*
Within:	0.04281	3	0.01427		

*Not significant at p < 0.05

Table D-3. ANCOVA of Weight Differences (g) of Earthworms in Soil Amended with CADS

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F Value	Significance Level
Total:	0.01042	10			
Between:	0.00628	3	0.00209	3.53765	0.07649*
Within:	0.00414	7	0.00059		

*Not significant at p < 0.05

Table D-4. Newman-Keuls Analysis of all Treatments, Pairwise, and Ranked from Low to High: Final Weights (g) of Earthworms Raised in Soil Amended with GD Simulant

Treatment	0.50%	0.10%	0%	1.0%	2.5%
			q values		
0.50%		0.548	0.913	3.652	5.112*
0.10%			0.365	3.104	4.564*
0.00%				2.739	4.199*
1.00%					1.461
2.50%					
q(95%)		3.15	3.88	4.33	4.65
q(99%)		4.48	5.27	5.77	6.14

*Significant at p < 0.05

Table D-5. Newman-Keuls Analysis of all Treatments Pairwise and Ranked from Low to High: Final Weights (g) of Earthworms Raised in Soil Amended with HL Simulant

Treatment	0.50%	1.0%	2.5%	0.10%	0%
			q values		
0.50%		0	0	4.307	7.711**
1.00%			0	4.307*	7.711**
2.50%				4.307*	7.711**
0.10%					3.404*
0.00%					
q(95%)		3.15	3.88	4.33	4.65
q(99%)		4.48	5.27	5.77	6.14

*Significant at $p < 0.05$
**Significant at $p < 0.01$

Table D-6. Newman-Keuls Analysis of all Treatments, Pairwise, and Ranked from Low to High: Final Weights (g) of Earthworms Raised in Soil Amended with CADS

Treatment	2.5%	0.50%	1.0%	0.10%	0%
			q values		
2.50%		18.036**	22.074**	22.612**	22.881**
0.50%			4.038*	4.576*	4.845*
1.00%				0.538	0.808
0.10%					0.269
0.00%					
q(95%)		3.15	3.88	4.33	4.65
q(99%)		4.48	5.27	5.77	6.14

*Significant at $p < 0.05$
**Significant at $p < 0.01$

QUALITY ASSURANCE

This study was examined for compliance with the Standard Operating Procedures governing the testing described below. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date reported
Earthworm dosing	2 Jun 1989	2 Jun 1989
Plant thinning	16 Jun 1989	16 Jun 1989
Final report and Data Audit	2 May 1990	2 May 1990

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Dennis W. Johnson 2 May 1990

DENNIS W. JOHNSON
Director, Quality Assurance Unit
Toxicology Division